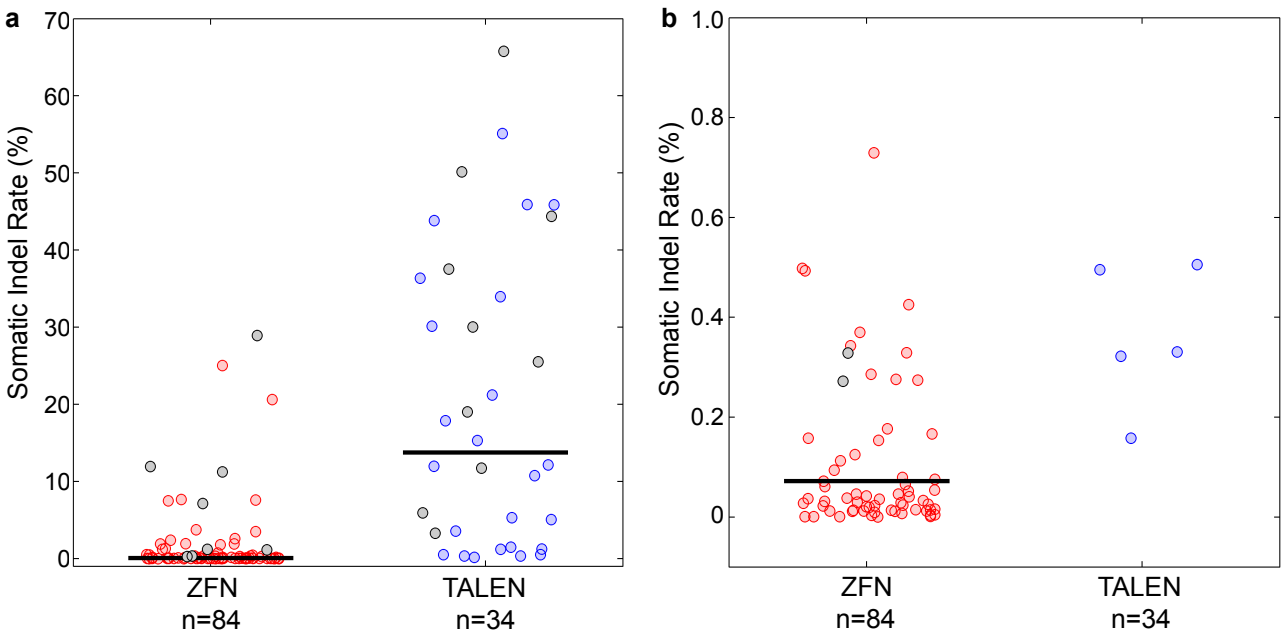
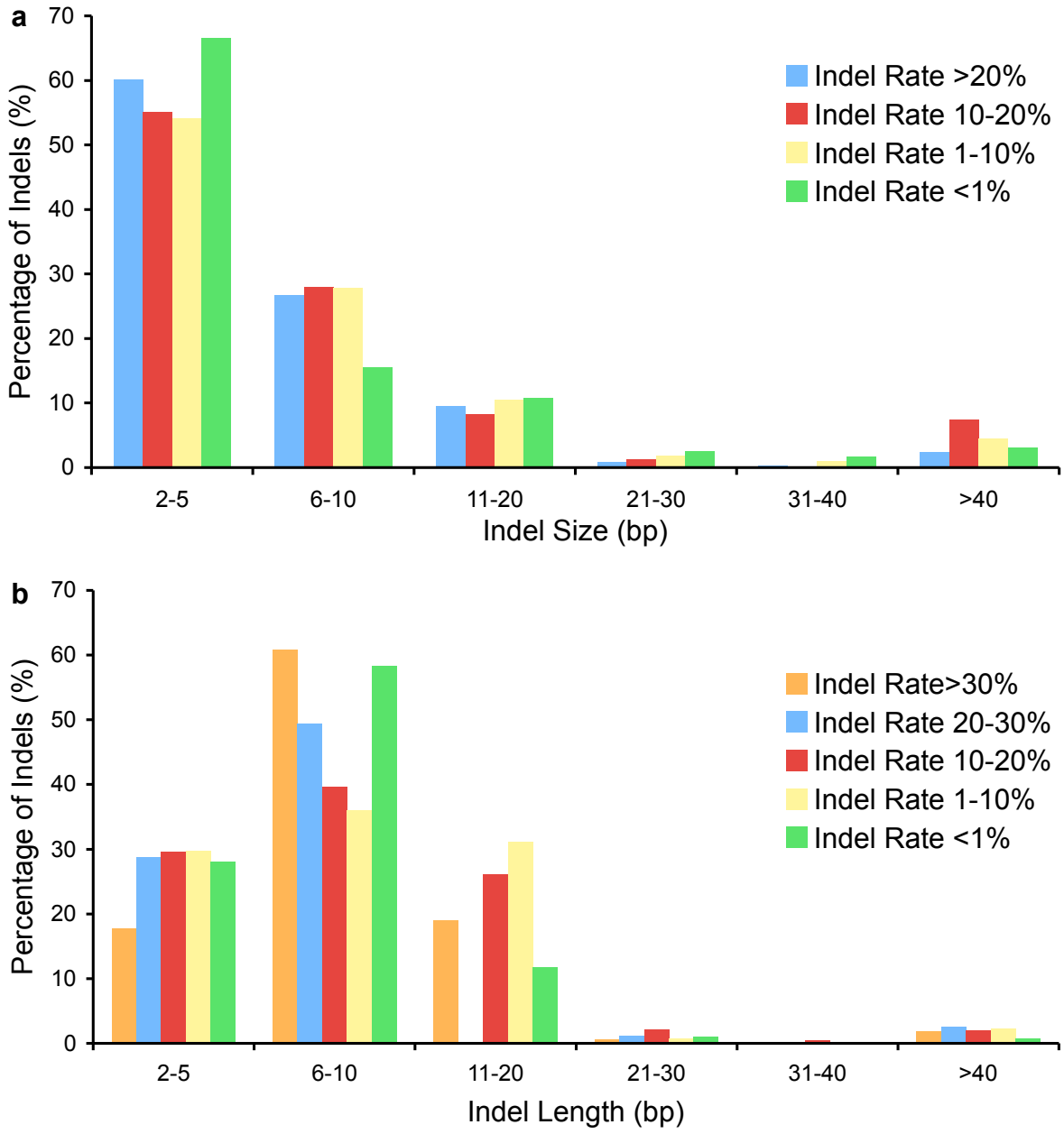
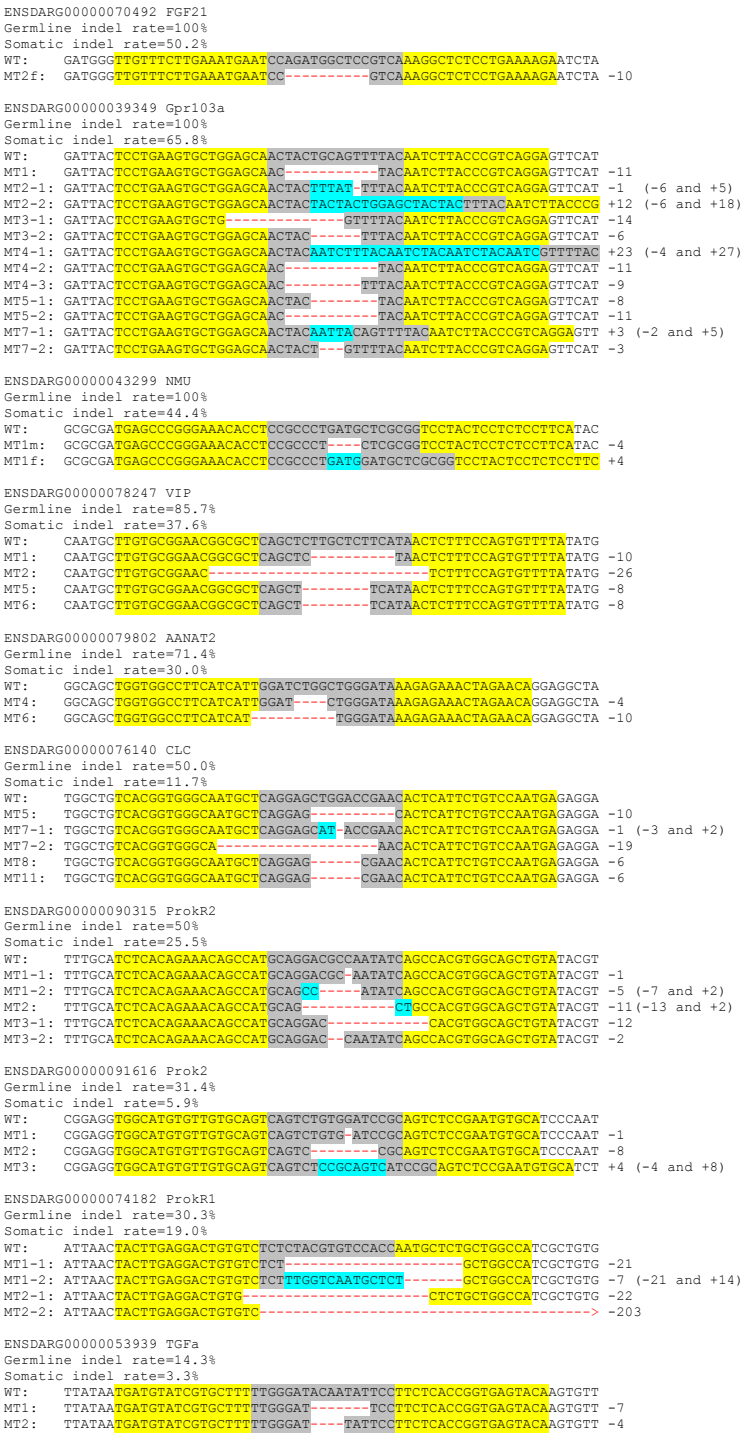


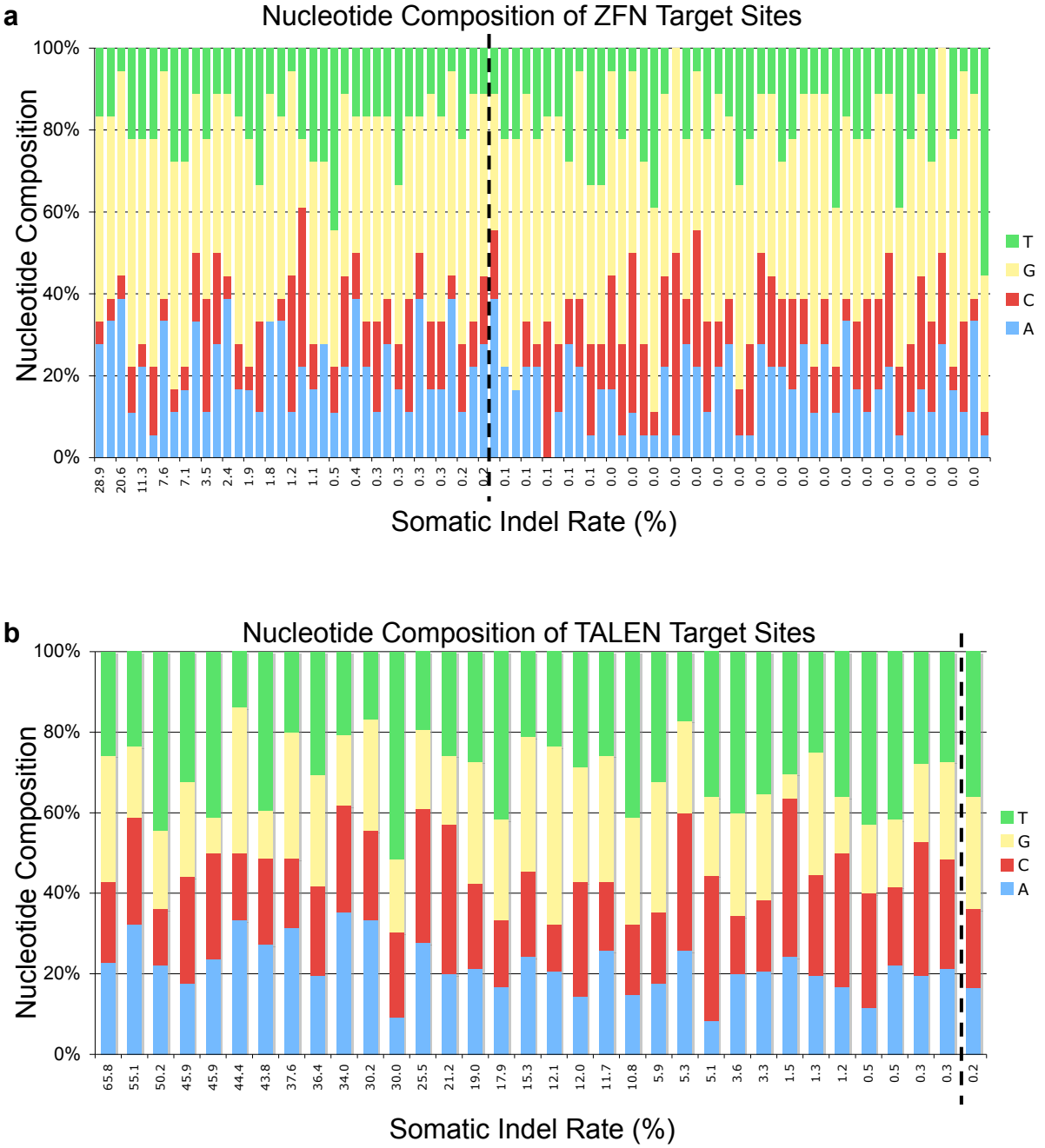
Supplementary Figure S1. TALENs induce higher somatic indel rates than ZFNs in zebrafish embryos. (**a**) Data points represent somatic indel rates for each of the 84 ZFN and 34 TALEN pairs tested. Black bars indicate median somatic indel rate for each type of nuclease. Indel rates were significantly higher for TALENs compared to ZFNs ($p=5.1 \times 10^{-12}$ using Wilcoxon rank sum test). Black data points represent ZFNs and TALENs for which germline mutants were generated. Panel (**b**) shows data for nucleases that induced somatic indels at rates up to 1%.



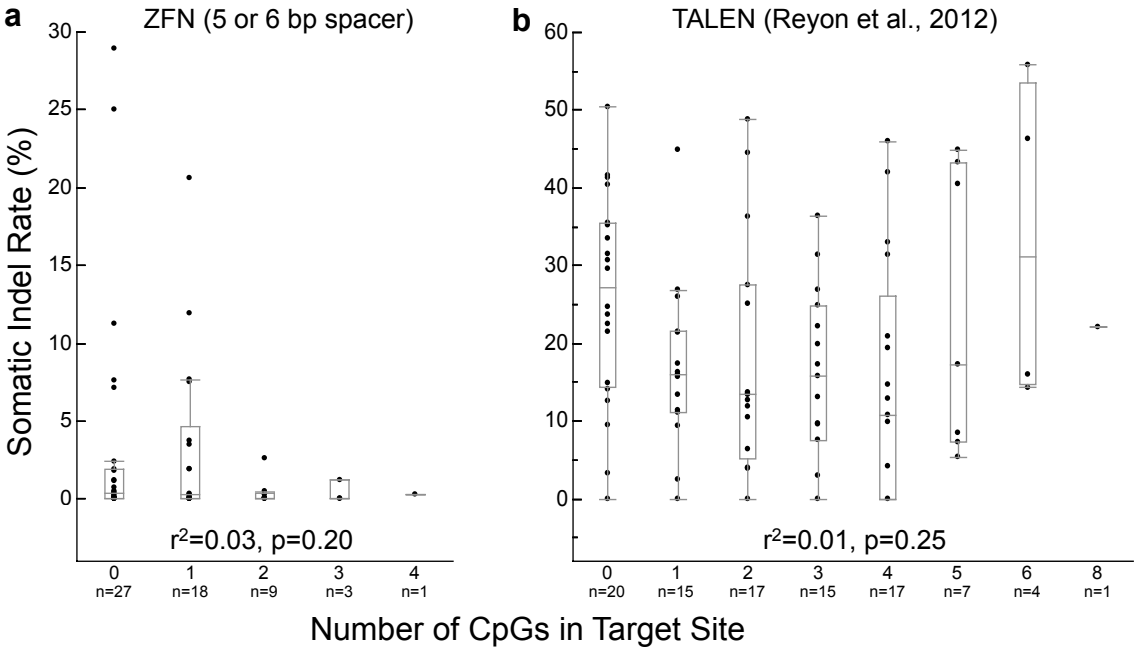
Supplementary Figure S2. The distribution of somatic indel size is similar for nucleases with different somatic indel rates. Somatic indels that were induced by ZFNs (**a**) and TALENs (**b**) have similar size distributions regardless of the indel rate. Median indel size and number of sequence reads are as follows. (**a**) Indel rate>20%: median=4, n=1,825,338; indel rate 10-20%: median=4, n=229,318; indel rate 1-10%: median=5, n=764,679; indel rate <1%: median=5, n=59,531. (**b**) Indel rate>30%: median=9, n=3,166,437; indel rate 20-30%: median=6, n=1,441,194; indel rate 10-20%: median=9, n=845,757; indel rate 1-10%: median=7, n=64,466; indel rate<1%: median=7, n=10,086.



Supplementary Figure S3. Sequences of TALEN-induced germline mutations. TALEN target sequences and spacer sequence are highlighted in yellow and grey, respectively. Deletions are indicated by red dashes and insertions are highlighted in blue. Only mutations that were analyzed using Sanger sequencing are shown.



Supplementary Figure S4. Nucleotide composition of ZFN and TALEN target sites used in this study. The nucleotide composition of ZFN (**a**) and TALEN (**b**) target sites, sorted in decreasing order of somatic indel rate, are shown. The T nucleotide that precedes all TALEN target half-sites is not included in the analysis. The vertical dashed lines separate active and inactive nucleases.



Supplementary Figure S5. Comparison of somatic mutation rate and target site CpG content. (**a**) Data points represent somatic indel rates at each of the 58 ZFN target sites that contained 5 or 6 bp spacers. Target sites containing 7 bp spacers were not included in this analysis. There is no correlation between mutation rate and CpG number (r^2=0.03, p=0.20 using Spearman's rank correlation). (**b**). Data points represent somatic indel rates for each of the 96 TALEN target sites reported by Reyon et al. 2012 (reference 41). Targets containing 0 CpGs tend to be mutated at slightly higher rates than those containing 1, 2, 3 or 4 CpGs, but the difference across all data points is not significant (r^2=0.01, p=0.25 using Spearman's rank correlation). The box within each plot indicates the middle half of the data and the line in each box indicates the median. The lines extending from the box indicate the farthest data point that is within 1.5 interquartile ranges from the 1st and 3rd quartiles. Individual data points outside these lines are possible outliers. N indicates number of target sites in a category.